REMARKS

Applicants thank the Examiner for the consideration given the present application. Claims 1-14 are pending, of which claim 1 is independent, and claims 10-14 are added. Support for added claims 10-14 can be found in the present specification, e.g., page 13, line 20, through page 14, line 7.

Applicants traverse and request reconsideration of the rejections under 35 U.S.C. §112, first and second paragraphs. The claims are supported by an enabling specification. As to claims **6 and 7**, attention is invited to the specification at pages 10, 13, and 14. Claim **7** is supported by the original specification.

Applicants traverse and request reconsideration of the rejection of claims 1-7 under 35 U.S.C. §102(b) as being anticipated by U.S. 6,187,456 to Lever as evidenced by the Rentz article, as well as the rejection of claims 8 and 9 under 35 U.S.C. §103(a) as being unpatentable over Lever in view of Rentz.

As noted in the Office Action, Lever describes an antimicrobial agent comprising a silver ion carrier such as silver zirconium phosphate, but fails to disclose a silver ion carrier having an anti-coronaviral effect such as the anti-SARS effect set forth in Applicants' claims 8 and 9.

The Office Action asserts the antimicrobial effects of the Lever compound will also necessarily have anti-coronaviral effects,

specifically, the anti-SARS effects evidenced by Rentz. However, on page 112, eight and ninth lines from the bottom of the page 112, Rentz reports that the "silver nitrate, silver sulfadiazine and electrolytically produced Ag^+ all had different antiviral properties". Thus, even taking Rentz into account, one skilled in the art would not expect an anti-coronaviral effect from a silver ion carrier such as silver zirconium phosphate, which is different from silver nitrate, silver sulfadiazine, and electrolytically produced Ag^+.

The Office Action contends Rentz refers to SARS in relation to electrolytically produced Ag^+ at page 113, fifth line from the bottom of the page, through page 114, fourth line from the bottom of the page. However, it is conventionally understood that the bactericidal activity of the silver zirconium phosphate is not derived from silver ions, but rather from superoxide or hydrogen peroxide formed by certain photochemical reactions catalyzed by the silver zirconium phosphate.

Attention is directed to the enclosed article by Kourai et al. (*Journal of Antibacterial Antifungal Agents*, Vol. 22, No. 10, 1994, pp. 595-601), especially the Abstract and Conclusions on pages 595 and 601.

The silver zirconium phosphate used in the presently claimed invention is a powdery substance that is different from electrolytically produced Ag^+ and other Ag-ion containing solutions disclosed by Rentz. In addition, the silver zirconium phosphate used in the presently claimed invention has nothing to do with the

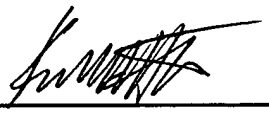
oligodynamic effect of electrolytically produced Ag^+ described in the accompanying article by Kourai et al. Accordingly, Applicants respectfully submit there is no scientific basis for combining Lever with Rentz.

In view of the foregoing amendments and remarks, Applicants believe their claims 1-14 define novel and unobvious inventions over the Lever and Rentz references, and withdrawal of the outstanding rejections are respectfully requested. The present application is in condition for allowance, and such action is urged.

To the extent necessary during prosecution, Applicants hereby request any required extension of time not otherwise requested and hereby authorize the Commissioner to charge any required fees not otherwise authorized, including application processing, extension, and extra claims fees, to Deposit Account 06-1135.

Respectfully submitted,

FITCH, EVEN, TABIN & FLANNERY



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【Original】

Mode of Bactericidal Action of Zirconium Phosphate Ceramics Containing Silver Ions in the Crystal Structure

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【Original】

Mode of Bactericidal Action of Zirconium Phosphate Ceramics Containing Silver Ions in the Crystal Structure

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NOVARON, a zirconium phosphate ceramic containing silver ions in the crystal structure, exhibits strong antimicrobial activity against gram positive and negative bacteria, yeasts and molds. In this study, we investigated the mode of bactericidal action of NOVARON using *Escherichia coli* K12 W3110 and *Staphylococcus aureus* IFO 12732. An aqueous suspension of 5,000 μg per ml of NOVARON was kept for 24 hr at 25℃ and filtered with a membrane filter (pore size : 0.2 μm). The filtrate did not exhibit bactericidal activity. Therefore, it was suggested that the bioactive materials which play a role in the bactericidal action are not silver ions but ceramics particles instead. NOVARON exhibited strong bactericidal activity in an atmosphere of oxygen, but the activity was extremely reduced in that of nitrogen. The bactericidal activity of NOVARON was approximately proportional to the intensity of irradiation with visible light. In particular, the activity was enhanced by the irradiation with a wave length region of about 650−780nm. In addition, the activity was extremely inhibited by the addition of catalase, superoxide dismutase or L−cysteine. When the aqueous suspension of NOVARON was irradiated with visible light separated with a diffraction grating, the formation of superoxide was approximately proportional to the wave length. Furthermore, a small amount of hydrogen peroxide in the aqueous suspension of NOVARON was detected when the suspension was irradiated with a white light in an atmosphere of oxygen or air. These findings lead to a conclusion that bactericidal active materials are superoxide and hydrogen peroxide formed by certain photochemical reactions on the surface of NOVARON particles.

(Accepted 13 April 1994)

Key words : Zirconium phosphate (リン酸ジルコニウム)/Ceramics sterilizer (セラミックス殺菌剤)/Bactericidal activity (殺菌活性)/Superoxide (活性酸素)/Hydrogen peroxide (過酸化水素)/Silver ion (銀イオン)

INTRODUCTION

The organometallic and inorganometallic compounds of group IB, IIB and IVB exhibit antimicrobial activities[1-8]. Zeolite containing silver ions has also shown strong bactericidal activity[9]. Montmorillonite supporting silver chelate of 2−(4−thiazolyl) benzimidazol has also exhibited strong antimicrobial activities[10]. Silver ions released from the zeolite or the montmorillonite particles probably play a role in the bactericidal action. Our previous paper[11] described the antimicrobial characteristics of zirconium phosphate ceramics contain-

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ing silver ions in the crystal structure against gram positive and negative bacteria, yeasts and molds. The ceramic pieces were white and cube-shaped and 0.3～0.5 μm in size. No silver ions were liberated from the ceramics by treatment with boiling water. The ceramics had strong bactericidal activity and fungicidal activity, but germicidal action was not observed against the spores of *Bacillus subtilis*. In addition, the bactericidal activity of the ceramics was proportional to the stirring speed of bacterial cell suspension.

Recently we found that the bactericidal activity of zirconium phosphate ceramics containing silver ion was extremely enhanced by white light irradiation and oxygen. The purpose of this investigation was to delineate the bactericidal active materials of NOVARON, and to elucidate the mode of the action against *Escherichia coli* K12 W3110 and *Staphylococcus aureus* IFO 12732.

MATERIALS AND METHODS

Chemicals and enzymes

The procedures of synthesis and chemical properties of zirconium phosphate ceramics containing silver ion, NOVARON, were previously described[1]. Hydrogen peroxide, L-cysteine, (±)-epinephrine, $AgNO_3$, $ZnCl_2$, $CuSO_4 \cdot 5H_2O$, and $MnCl_2 \cdot 4H_2O$ were purchased from Wako Pure Chemical Industries, Ltd., Osaka Japan. Enzymes such as catalase (EC 1.11.1.6) from bovine liver and superoxide dismutase (EC 1.15.1.1) from bovine erythrocyte were also purchased from Wako Pure Chemical Industries.

Minimum bactericidal concentration

Staphylococcus aureus IFO 12732 and *Escherichia coli* K12 W3110 were used for the measurement of minimum bactericidal concentration (MBC). Each bacterium preincubated in a nutrient broth (Difco Lab., USA) for 18 hr at 37℃ was inoculated into a fresh nutrient broth. After the inoculated culture was grown to early log-phase, the cells were harvested by centrifugation (5,000×g, 10min) at 2℃. The harvested cells were washed twice with an ice-cold sterilized water and suspended in an ice-cold sterilized water at 10^6 cells per ml. The aqueous suspension of NOVARON (1 mg per ml) was diluted stepwise with sterilized water. Each 1 ml portion of the diluted suspension was mixed with 1 ml of the washed cell suspension and incubated in a water bath shaker for 30 min at 30℃. Then the 0.1ml portions of the mixtures were withdrawn and inoculated into 2 ml of a fresh nutrient broth. After the inoculated broth was incubated at 37℃ for 24 hr, MBC was determined by visual inspection. The MBCs of Ag^+ ion, Zn^{2+} ion, Cu^{2+} ion, Mn^{2+} ion, and hydrogen peroxide were also measured in a similar manner described above.

Bactericidal activity

The exponential-phase cells of *Escherichia coli* K12 W3110 were employed for bactericidal tests. Ten-ml portions of the washed cell suspension (10^6 cells per ml) were poured into 100ml flasks containing prescribed amounts of NOVARON and the flask contents were incubated on a reciprocal flask shaker (120 stroke per min) at 30℃. After 5, 10, 20 and 30min, 0.5ml portions of the contents were withdrawn and the appropriate decimal dilution was made with a sterilized physiological saline, followed by spreading the respective diluted suspension on nutrient agar plates. The plates were incubated at 37℃ for 24 hr, then viable cell colonies were counted.

Effect of oxygen or nitrogen on bactericidal activity of NOVARON

The mixture of 10ml of the washed log-phase cell suspension (10^6 cells per ml) of *E. coli* K12 W3110 and prescribed amounts of NOVARON in 100ml Erlenmeyer flask equipped with a gas-inlet tube was incubated on a reciprocal flask shaker (120 stroke per min) at 30℃ in an atmosphere of nitrogen, oxygen or air under white light irradiation with a 300watt incandescent bulb or in a dark room. After 5, 10, 20, and 30min, 0.5ml portions of the contents were withdrawn and the viable cells in the contents were counted as mentioned above.

Effect of white light irradiation on bactericidal activity

The washed log-phase cell suspension (10^6 cells per ml) of *E. coli* K12 was treated with 20 μg per ml of NOVARON under white light irradiation with a 300 watt incandescent bulb for 30min at 30℃ on a reciprocal shaker (140 stroke per min). The dis-

tance between the bulb and the cell suspension was 0.1, 0.3, 0.5, 1.0, 1.5 or 2.0m. After 5, 10, 20 and 30 min, 0.5ml portions of the contents were withdrawn and the viable cells in the contents were counted as mentioned above.

Effect of catalase, superoxide dismutase or L-cysteine on bactericidal activity

The washed log-phase cell suspension (10^6 cells per ml) of *E. coli* K12 containing 2,000 units per ml of catalase, 330 units per ml of superoxide dismutase or 200 μg per ml of L-cysteine was treated with 10 μg per ml of NOVARON under room light at 30℃. After 5, 10, 20 and 30min, 0.5ml portions of the cell suspensions were withdrawn and the viable cells in the contents were counted as mentioned above.

Effect of wave length of iradiation light on bactericidal activity

Three-ml portions of log-phase cell suspensions (10^6 cells per ml) of *E. coli* K12 W3110 and *S. aureus* IFO 12732 were poured into quartz cuvettes containing 30 μg of NOVARON respectively. The contents of the cuvettes were irradiated with separated visible light (400~780nm) with a fluorescence spectrophotometer (HITACHI 204) under bubbling sterilized air at 30℃ for 60min. The viable cells in the contents were measured by a colony count method as described above.

Measurement of superoxide

Epinephrine is quantitatively oxidized by superoxide to give adrenochrome[12-14]. The mixture of 70 μg of NOVARON and 3.5ml of 1 mM epinephrine in 0.02 M phosphate buffer (pH 7.0) containing 0.1mM of EDTA was irradiated with separated visible light (400~800nm) with a HITACHI 204 fluorescence spectrophotometer under stirring at 20℃. After the mixture was filtered with a membrane filter (pore size 0.2 μm), the adrenochrome in the filtrate was measured with a Shimadzu UV-160 spectrophotometer at 480nm.

Measurement of hydrogen peroxide

Hydrogen peroxide oxidizes iodide ion to give iodine that reacts with an aqueous starch solution. The mixture of 10ml of 0.05M phosphate buffer (pH 4, 7 and 9) containing 50mg of NOVARON and 0.2 ml KI-starch aqueous solution was allowed to stand under white light irradiation with a 300 watt incandescent bulb or in a dark room for 30min at 30℃ in an atmosphere of air, oxygen or nitrogen. The distance between the bulb and the mixture was 0.5m. Hydrogen peroxide was determined with a Shimadzu UV-160 spectrophotometer at 536nm.

RESULTS AND DISCUSSION

Minimum bactericidal concentration of metal ions and NOVARON

In a previous paper[11], we have shown that the bactericidal activity of NOVARON varies in proportion to the stirring speed of bacterial cell suspension. The result indicates that the surface of NOVARON may play a role in the bactericidal action or that the active materials may exist in the neighborhood of the particle surface. To investigate the bioactive materials on the surface of NOVARON particles, the MBCs of NOVARON and the supernatant of NOVARON aqueous suspension, Ag^+ ion, Zn^{2+} ion, Cu^{2+} ion, and Mn^{2+} ion were measured against *E. coli* K12 W3110 and *S. aureus* IFO 12732 (Table 1). MBCs of NOVARON against *E. coli* K12 W3110 and *S. aureus* IFO 12732 were 15.6 μg per ml and 125 μg per ml respectively. The aqueous suspension of 5 mg per ml of NOVARON was kept for 24 hr at 25℃ and filtered with a membrane filter. The filtrate exhibited no

Table 1. Minimum bactericidal concentration (MBC) of metal ions, NOVARON and filtrate of NOVARON suspension against bacteria

Materials	MBCs (μg/ml)[a]	
	Escherichia coli K12 W3110	*Staphylococcus aureus* IFO 12732
Filtrate[b]	>2,500[c]	>2,500[c]
NOVARON	15.6	125
Ag^+	0.00024	0.0078
Zn^{2+}	3.9	62.5
Cu^{2+}	>2,000	500
Mn^{2+}	>2,000	>2,000

[a] MBCs were measured by a dilution method at 30℃ for 30 min.
[b] NOVARON suspension (5,000 μg/ml) in a distilled water was kept for 24 hr at 25℃ and filtered with a membrane filter.
[c] Concentration based on original suspension of NOVARON.

bactericidal activity. On the other hand, Ag^{+} ion exhibited much stronger activity than Cu^{2+} ion, Zn^{2+} ion, Mn^{2+} ion, and NOVARON. It was reasoned that the filtrate of NOVARON must exhibit strong bactericidal activity if silver ions are actually released from NOVARON particles. From these findings, it was indicated that the bioactive materials of NOVARON that play a role in the bactericidal action are not silver ions but other materials.

Effect of white light irradiation, nitrogen and oxygen on bactericidal activity of NOVARON

To study the characteristics of bactericidal action of NOVARON, the effect of white light irradiation, nitrogen and oxygen on the bactericidal activity against log-phase cells of *E. coli* K12 was investigated. Ten μg per ml of NOVARON did not exhibit bactericidal activity in an atmosphere of purified nitrogen in a dark room, while under white light irradiation with a 300 watt incandescent bulb it exhibited weak bactericidal activity (Fig. 1). On the other hand, the bactericidal activity of 10 μg per ml of NOVARON was extremely enhanced by the irradiation of white light in an atmosphere of oxygen

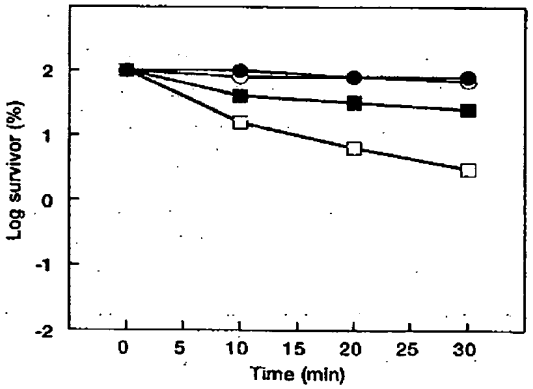

Fig.1. Effect of white light irradiation on the bactericidal activity of NOVARON against *E. coli* K12 W3110 in an atmosphere of nitrogen. The log-phase cell suspension (10^6 cells/ml) was treated with NOVARON under white light irradiation with a 300 watt incandescent bulb for 30 min at 30℃ on a reciprocal shaker (120 strokes/min). The distance between the flask and the bulb was 50cm. Symbols : ○, 0 μg/ml NOVARON under white light irradiation ; ●, 0 μg/ml NOVARON in a dark room ; □, 10 μg/ml NOVARON under white light irradiation ; ■, 10 μg/ml NOVARON in a dark room.

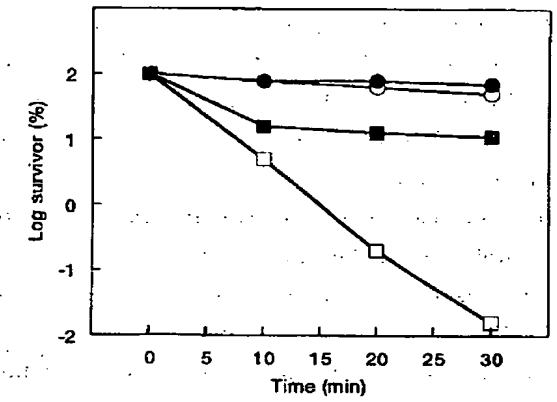

Fig.2. Effect of white light irradiation on the batericidal activity of NOVARON against *E. coli* K12 W3110 in an atmosphere of oxygen.
The log-phase cell suspension (10^6 cells/ml) was treated with NOVARON under white light irradiation with a 300 watt incandescent bulb for 30 min at 30℃ on a reciprocal shaker (120 strokes/min). The distance between the flask and the bulb was 50 cm. Symbols : ○, 0 μg/ml NOVARON under white light irradiation ; ●, 0 μg/ml NOVARON in a dark room ; □, 10 μg/ml NOVARON under white light irradiation ; ■, 10 μg/ml NOVARON in a dark room.

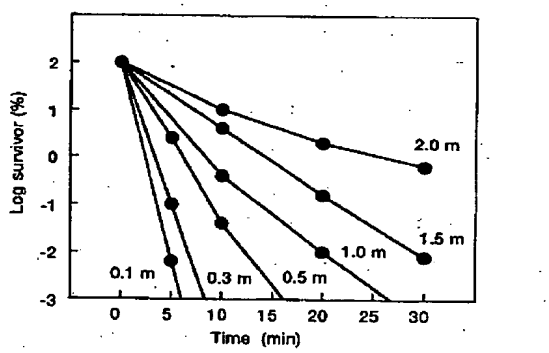

Fig.3. Effect of the intensity of white light irradiation on the bactericidal activity of NOVARON against *E. coli* K12 W3110 in the atmosphere.
The log-phase cell suspension (10^6 cells/ml) was treated with NOVARON (20 μg/ml) under white light irradiation with a 300 watt incandescent bulb for 30 min at 30℃ on a reciprocal shaker (140 strokes/min). The numerical values indicate the distance between the bulb and the cell suspension.

(Fig. 2). These results suggest that the bactericidal action of NOVARON requires oxygen and light. To verify that the bactericidal action of NOVARON needs light, the effect of the intensity of white light irradiation on the bactericidal activity was investigated in an atmosphere of air. The cell suspension (10^6

cells/ml) of *E. coli* K12 was treated with 20 μg per ml of NOVARON under white light irradiation at 30 ℃ for 30min under stirring with a reciprocal shaker (120 stroke per min). The distance between a 300 watt incandescent bulb and the flask containing cell suspension was 0.1, 0.3, 0.5 1.0, 1.5 or 2.0 meters. The bactericidal activity of NOVARON increased with decrease in the distance between the bulb and the flask (Fig. 3). It seems that the rate of the bactericidal action is proportional to the light intensity. When the distance was less than 0.3 meter, the viable cells were not detected. The results indicate that photochemical reactions on the surface of NOVARON occur by white light irradiation and that a bioactive material such as superoxide is formed.

Effect of catalase, superoxide dismutase or cysteine on bactericidal activity

To delineate the bioactive materials on the surface of NOVARON, the effect of the addition of catalase, superoxide dismutase or L-cysteine on the bactericidal activity was investigated under white light irradiation. Fig. 4 shows how catalase or superoxide dismutase affected the bactericidal activity of NOVARON against log-phase cells of *E. coli* K12 at 30 ℃. The bactericidal activity of NOVARON was reduced by the addition of 2,000 units per ml of catalase or 330units per ml of superoxide dismutase. On the other hand, the mixture of 2,000 units per ml of catalase and 330 units per ml of superoxide dismutase, which was denatured by heating at 90℃ for 15 min, did not inhibit the bactericidal activity.

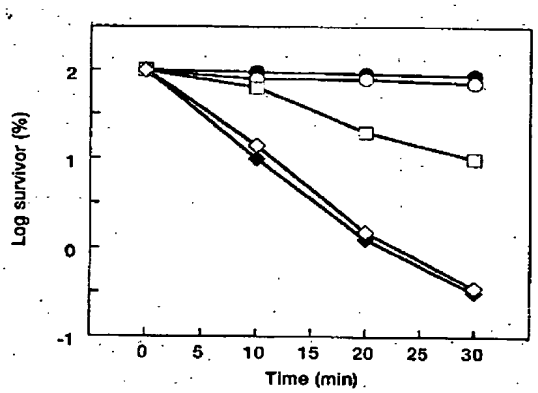

Fig.4. Effect of catalase and superoxide dismutase on the bactericidal activity of NOVARON against *E. coli* K12 W3110. The log-phase cell suspension (10^6 cells/ml) containing catalase or superoxide dismutase was treated with NOVARON under room light. Symbols : ●, 0 μg/ml NOVARON ; ◆, 10 μg/ml NOVARON ; □, 10 μg/ml + 330 units/ml superoxide dismutase ; ○, 10 μg/ml NOVARON + 2,000 units/ml catalase ◇, 10 μg/ml NOVARON + 330 units/ml denatured superoxide dismutase by heating+2,000 units/ml denatured catalase by heating.

In a previous paper[11], we have shown that the bactericidal activity of NOVARON was considerably inhibited by the addition of peptone. These findings imply that the antagonistic action of catalase and superoxide dismutase against the bactericidal action of NOVARON is not due to the effect of the enzyme protein but their enzymatic actions. In addition, the bactericidal activity was strongly inhibited by the addition of 200 μg per ml of L-cysteine (Fig. 5). Consequently, it was suggested that bioactive materials formed on the surface of NOVARON by white light irradiation are hydrogen peroxide and superoxide.

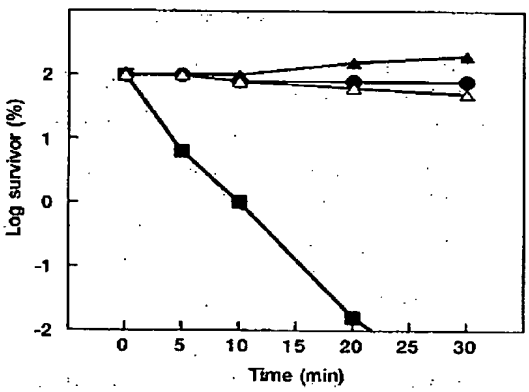

Fig.5. Effect of L-cysteine on the bactericidal activity of NOVARON against *E. coli* K12 W3110. The log-phase cell suspension (10^6 cells/ml) containing L-cysteine with NOVARON under room light. Symbols : ●, 0 μg/ml NOVARON ; ■, 20 μg/ml NOVARON ; △, 20 μg/ml NOVARON+200 μg/ml L-cysteine ; ▲, 200 μg/ml L-cysteine.

Effect of wave length of irradiation light on superoxide production

Epinephrine is quantitatively oxidized by superoxide to give adrenochrome. The effect of wave length of irradiation light on superoxide production was investigated concerning the formation of adrenochrome. The mixture of 70 μg of NOVARON and 3.5ml

of 1 mM epinephrine in 0.02M phosphate buffer (pH 7.0) containing 0.1mM of EDTA was irradiated with visible light at wavelengths of 400–800nm while being stirred 20℃. The adrenochrome in the filtrate of the mixture with a membrane filter was measured at 480nm. The absorbance of adrenochrome increased with an increase in irradiated wave length (Fig. 6). The results suggest that the amounts of superoxide formation were strongly affected by the wave length of the irradiation light.

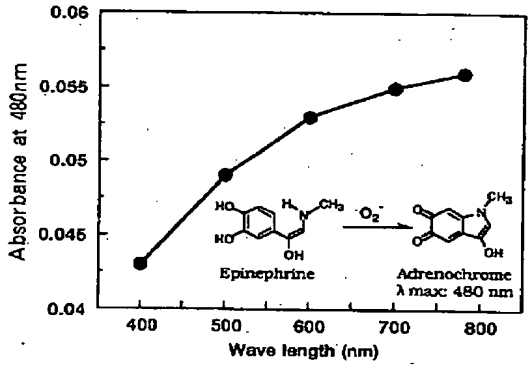

Fig.6. Effect of wave length of irradiation light on the superoxide formation of NOVARON. The mixture of 3.5ml of 1mM epinephrine in 0.02M phosphate buffer (pH 7) containing 0.1mM EDTA and 70 μg of NOVARON was irradiated with visible light of wave lengths 400~800nm with a fluorescence spectrophotometer for 60min under stirring at 20℃. After the mixture was filtered with a membrane filter (pore size 0.2 μm), the adrenochrome in the filtrate was measured with a spectrophotometer at 480nm.

Effect of wave length of irradiation light on bactericidal activity

The effect of wave length of the irradiation light on the bactericidal activity of NOVARON was investigated using log-phase cells of *E. coli* K12 W3110 and *S. aureus* IFO 12732. Each cell suspension containing 10 μg per ml of NOVARON was irradiated with the visible light at wave lengths of 400–780 nm with a fluorescence spectrophotometer under bubbling sterilized air at 30℃ for 60min. The viable cells of *E. coli* K12 and *S. aureus* were decreased with an increase in irradiated wave length (Fig. 7). These results are consistent with the view that the formation of superoxide increases with an increase in wave length of illuminated light.

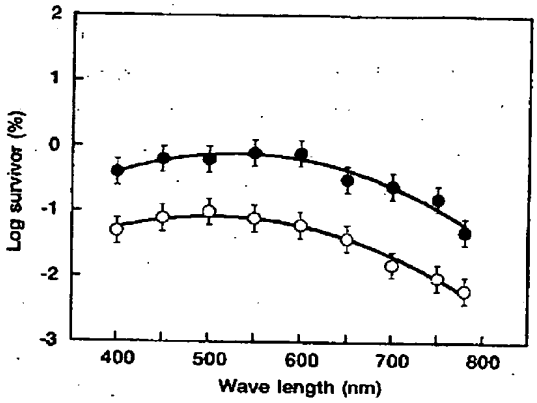

Fig.7. Effect of wave length of irradiation light on the bactericidal activity of NOVARON against *E. coli* K12 W3110 and *S. aureus* IFO 12732. The log-phase cell suspensions (10^6 cells/ml) containing 10 μg/ml of NOVARON were irradiated with visible light at wave length of 400~780nm with a fluorescence spectrophotometer for 60 min under bubbling sterilized air. Symbols : ○, *E. coli* K12 W3110 ; ●, *S. aureus* IFO 12732.

Hydrogen peroxide

To determine the formation of hydrogen peroxide, the mixture of 10ml of 0.05M phosphate buffer (pH 4, 7 and 9) containing 50mg of NOVARON and 0.2ml KI-starch aqueous solution was allowed to stand under white light irradiation with a 300 watt incandescent bulb or dark room for 30min at 30℃ under an atmosphere of air, oxygen or nitrogen. Hydrogen peroxide was determined by a photometric measurement at 536nm. A small amount of hydrogen peroxide was detected at pH 4 and 7 in an atmosphere of oxygen and air when the mixture was irradiated with white light as shown in Table 2. On the other hand, hydrogen peroxide was scarcely detected at pH 9 in a dark room. In addition, NOVARON has a weak catalase-like activity at pH values showing weak alkalinity. Because of the catalse-like activity, it seems that the formation of hydrogen peroxide is not detected in a weak alkaline solution.

Bactericidal activity of hydrogen peroxide

MBCs of hydrogen peroxide against *E. coli* K12 W3110 and *S. aureus* IFO 12732 were measured (Table 3). The activity of hydrogen peroxide was considerably low (MBC : 300 μg per ml). MBCs of NOVARON against *E. coli* K12 W3110 and *S. aureus* IFO 12732 were 15.6 μg per ml and 125 μg per ml respectively described above. Therefore, in view of

Table 2. Generation of hydrogen peroxide by NOVARON

Atmosphere	White light irradiation[a]	Hydrogen peroxide[b] pH 4	pH 7	pH 9
Air	Irradiated	+[c]	+	−
	Dark room	−	−	−
Oxygen	Irradiated	++	+	−
	Dark room	−	−	−
Nitrogen	Irradiated	−	−	−
	Dark room	−	−	−

[a] The mixture of 10 ml of 0.05M phosphate buffer containing 50 mg of NOVARON and 0.2 ml KI/starch solution was allowed to stand under white light irradiation with a 300 watt incandescent bulb or in a dark room for 30 min at 30°C under an atmosphere of air, oxygen or nitrogen.

[b] Generation of hydrogen peroxide was determined with by a colorimetry at 536 nm.

[c] Symbols : −, <0.05 ppm ; +, 0.05~0.2 ppm ; ++, >0.2 ppm

Table 3. Minimum bactericidal concentration (MBC) of hydrogen peroxide against Bacteria

No.	Bacteria	MBCs (μg/ml)[a]
1	*Escherichia coli* K12 W3110	300
2	*Staphylococcus aureus* IFO 12732	300

[a] MBCs were measured by a dilution method at 30°C for 30 min.

these facts, the bioactive material which plays a major role in the bactericidal action seems to be superoxide. The hydrogen peroxide formed on the surface of NOVARON and its degradation product such as hydroxyl radical may play an important role in bactericidal actions if the hydrogen peroxide is immediately decomposed by a catalase-like activity on the surface into water and oxygen. Further work is needed to determine the detailed mode of bactericidal action.

CONCLUSIONS

1) The filtrate of the aqueous suspension of NOVARON did not exhibit bactericidal activity. Therefore, the bioactive materials that play a major role in the bactericidal action are not silver ions.

2) The bactericidal activity of NOVARON was proportional to the intensity of visible light. In particular, the activity was enhanced by the irradiation of wave length region of about 650 −780 nm.

3) The bactericidal activity of NOVARON was extremely inhibited by the addition of catalase, superoxide dismutase or L-cysteine.

4) When the aqueous suspension of NOVARON was irradiated with visible light, the formation of superoxide in the suspension was proportional to the wave length.

5) A small amount of hydrogen peroxide in the aqueous suspension of NOVARON was detected when the suspension was irradiated with a white light under an atmosphere of oxygen or air.

6) These findings lead to a conclusion that the bioactive materials that play a role in the bactericidal action are superoxide and hydrogen peroxide formed by certain photochemical reactions.

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